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Multiphoton Microscopy of Oxygen

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Molecular oxygen plays a unique role in cellular energy metabolism by serving as the terminal electron acceptor in the mitochondrial respiratory chain. Quantitative imaging of oxygen can provide invaluable information about metabolism in normal and diseased states. Two-photon phosphorescence lifetime microscopy (2PLM) enables measurements of oxygen concentration gradients in 3D with micron-scale resolution. Here we will discuss the principles of 2PLM and focus on the design of the oxygen probes, in which high two-photon absorption cross-sections are imparted by way of manipulating hidden *gerade* states in fully centrosymmetric phosphorescent metalloporphyrins. We further consider three-photon absorptions in these porphyrins and identify channels and states that interfere constructively or destructively in the excitation process. The experimentally measured three-photon absorption cross-sections of some of these porphyrins are among the highest reported to date for the 1700 nm tissue transparency window.